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BIODIESEL PRODUCTION USING *CHLORELLA VULGARIS* CULTIVATED IN INDUSTRIAL DAIRY WASTEWATER

Produksi dan Penilaian Biodiesel Menggunakan Chlorella vulgaris yang Dibudidayakan Dalam Air Limbah Industri Susu

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ABSTRACT

In this study, a method for producing third-generation biodiesel from *Chlorella vulgaris* (*C. vulgaris*) cultivated in dairy waste water is reported. The lipids were removed from the biomass, and biodiesel was produced through transesterification. The biodiesel was evaluated based on ASTM and EN standards, including density, viscosity, heating value, and flash point. Gas chromatography was used to determine the content, composition, and type of fatty acid methyl esters. The results revealed a 93% biodiesel yield with a composition of 33% saturated, 28% monounsaturated, and 37% polyunsaturated. Engine testing was conducted to monitor CO, CO_2, NOx, and smoke emissions, as well as assess biodiesel performance. The findings show that *C. vulgaris* cultivated in dairy wastewater can be used to produce third-generation biodiesel. The proposed method valorizes dairy waste water and might serve as an appropriate replacement for biodiesel generation and wastewater disposal.

Keywords: Biodiesel; *C. vulgaris*; Dairy Wastewater; Microalgae

ABSTRAK

Dalam penelitian ini, dilaporkan metode untuk memperoleh biodiesel generasi ketiga dari *Chlorella vulgaris* (*C. vulgaris*) yang dibudidayakan dalam air limbah susu. Lipid diambil dari biomassa, dan biodiesel diproduksi melalui transesterifikasi. Biodiesel dievaluasi berdasarkan standar ASTM dan EN, termasuk densitas, viskositas, nilai kalor, dan titik nyala. Kromatografi gas digunakan untuk menentukan kandungan, komposisi, dan jenis ester metil asam lemak. Hasil penelitian menunjukkan hasil biodiesel sebesar 93% dengan komposisi 33% jenuh, 28% tak jenuh tunggal, dan 37% tak jenuh ganda. Uji mesin dilakukan untuk memantau emisi CO, CO_2, NOx, dan asap, serta menilai kinerja biodiesel. Temuan menunjukkan bahwa *C. vulgaris* yang dibudidayakan dalam limbah susu dapat digunakan untuk memproduksi biodiesel generasi ketiga. Metode yang diusulkan memanfaatkan limbah air susu dan mungkin dapat menjadi pengganti yang tepat untuk produksi biodiesel dan pembuangan air limbah.

Kata kunci: Air Limbah Susu; *C. vulgaris*; Biodiesel; Mikroalga

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1. INTRODUCTION

Water pollution from dairy industries represents a serious environmental challenge in many countries, primarily due to the high pollutant load discharged in effluents and the large volumes of water required during processing (Raghunath *et al.* 2016; Choudhury *et al.* 2021). Dairy wastewater is typically rich in lipids, proteins, carbohydrates, and significant concentrations of nitrogen and phosphorus compounds. On average, the dairy sector generates between two and five liters of wastewater per liter of processed milk product (Dongre *et al.* 2020).

In recent years, increasing attention has been directed toward the use of microalgae for wastewater treatment across various industrial sectors, due to their ability to remove both organic and inorganic contaminants You *et al.* 2022). Most existing studies focus primarily on microalgae as a nature-based or phytoremediation technology aimed at pollutant removal, particularly for metals and hazardous substances. However, a significant research gap remains in integrating wastewater treatment with the simultaneous production of value-added bioproducts under optimized and scalable conditions, especially in the context of dairy effluents. Over the last decade, the concept of using wastewater as a cultivation medium for microalgae has emerged as a promising strategy to simultaneously address environmental remediation and resource recovery. Microalgal biomass can be further exploited for the production of high-value compounds with applications in pharmaceuticals, cosmetics, and the food industry (Gramegna *et al.* 2020). In this framework, microalgae offer a dual benefit: reducing the environmental burden associated with dairy wastewater disposal while enabling waste valorization into economically relevant products (Silva *et al.* 2018). Nevertheless, limited studies have comprehensively evaluated the full process chain—from wastewater treatment to downstream biofuel production and fuel quality assessment—within a single, integrated system.

Microalgae possess the ability to assimilate organic pollutants and convert them into cellular constituents such as lipids and carbohydrates. Unlike conventional bioremediation systems based on vascular plants, which primarily target inorganic compounds, microalgae can simultaneously utilize both organic and inorganic nutrients, enhancing treatment efficiency. Furthermore, microalgal biomass can contain up to 70–80% lipids (dry weight under optimized conditions), making it a highly promising feedstock for biofuel production (Brennan and Owende, 2010; Wang *et al.* 2010; Chisti 2007; Ratomski and Hawrot-Paw, 2021). Microalgae have been widely recognized as a key resource for third-generation biofuels (Mata *et al.* 2010; Chen *et al.* 2018), addressing many limitations associated with first- and second-generation biofuels, such as land use competition, high water demand, and climate dependency. Among various species, *Chlorella vulgaris* has attracted particular attention due to its high lipid productivity and adaptability to diverse environmental conditions (Hosseini and Shariati, 2009; Tao *et al.* 2017). Additionally, *C. vulgaris* is widely used in food, nutraceutical, and pharmaceutical applications, with documented health benefits including antioxidant, anti-inflammatory, and lipid-lowering effects (Gouveia *et al.* 2007; Galasso *et al.* 2019; Martins *et al.* 2021). Despite these advantages, there is still a lack of studies that systematically investigate the coupling of dairy wastewater treatment with lipid extraction, biodiesel production, and compliance with international fuel standards (EN and ASTM), particularly using *C. vulgaris* under realistic cultivation conditions.

To achieve high biomass productivity and lipid accumulation, cultivation parameters such as nutrient availability, temperature, and light must be carefully optimized. Although synthetic media are commonly employed, their high cost limits large-scale applications. Consequently, the use of alternative nutrient sources, including agricultural and industrial wastewaters, has gained increasing interest as a sustainable and cost-effective solution.

The novelty of this study lies in the development of an integrated and circular approach that simultaneously addresses wastewater remediation, biomass production, and biofuel generation using dairy wastewater as a growth medium for *Chlorella vulgaris*. Specifically, this work not only evaluates microalgal growth performance and nutrient removal efficiency but also extends to lipid extraction, biodiesel production, and detailed physicochemical characterization in accordance with international standards. Thus, this study provides a comprehensive framework that bridges the gap between laboratory-scale wastewater treatment and practical biofuel production systems. The primary objective is to demonstrate a sustainable pathway for converting dairy wastewater into valuable bioenergy resources, with a possible reduction in environmental impact and promoting waste valorization.

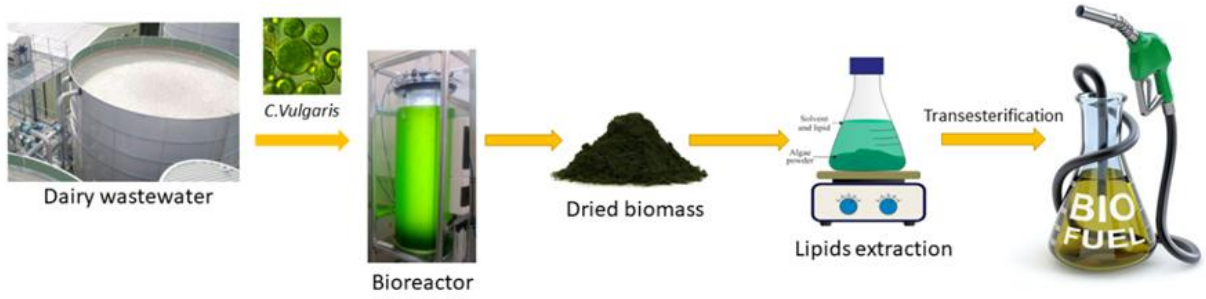


Figure 1. Schematic representation of the full process production of 3rd generation biodiesel.

2. METHODS

Physicochemical Characteristics of Wastewater

A local dairy industry provided wastewater in glass bottles that had not been purified. To avoid bacterial contamination, the solid particles were filtered with filter paper before being sterilized in an autoclave for 30 minutes. The pH together with the NH_3, NO_3 and PO_4 content in the wastewater were measured.

The concentrations of ammoniacal nitrogen (NH_3-N), nitrate nitrogen (NO_3^--N), and orthophosphate (PO_4^{3-}-P) in were determined using standard spectrophotometric methods.

Ammonium nitrogen (NH_3-N/NH_4^+) was quantified using the indophenol blue method (Utomo *et al.* 2022). In this procedure, ammonium reacts under alkaline conditions with hypochlorite and phenol in the presence of sodium nitroprusside as a catalyst to form indophenol blue. The absorbance was measured at 640–660 nm using a UV–Vis spectrophotometer, and concentrations were determined from a calibration curve prepared with ammonium chloride standards.

Nitrate nitrogen (NO_3^-- N) was determined by UV spectrophotometry at 220 nm, with correction at 275 nm to account for interference from dissolved organic matter (Hu *et al.* 2022).

Orthophosphate (PO_4^{3-}- P) was measured using the ascorbic acid molybdenum blue method (Barman *et al.* 2024). Under acidic conditions, orthophosphate reacts with ammonium molybdate and antimony potassium tartrate to form phosphomolybdic acid, which is subsequently reduced by ascorbic acid to form a blue complex. The absorbance was recorded at 880 nm, and concentrations were calculated using external calibration standards.

All measurements were performed in triplicate, and appropriate blanks and dilution factors were applied to account for matrix effects associated with dairy wastewater.

Microalgae Strain and Cultivation in Dairy Wastewater

The cultivation of *C. vulgaris* strain was carried out for two weeks in Erlenmeyer flasks using BG11 medium and stored in an incubator at 25 °C and under a fluorescent lamp. The oxygen was provided by an air-compressed system with a CO_2 concentration of 5%. As dairy wastewater contains organic carbon leading a mixotrophic growth which requires a reduction in CO_2 external demand compared (Abreu 2012). . Four dilutions of the diary wastewater in distilled water (10, 24, 50, and 75 % v/v) were prepared and sterilized in an autoclave at 120°C for 30 minutes prior the use. Afterward, 25 × 10^6 cells/ml of *C. vulgaris* were added to each dilution and exposed to 3500 Lux at 25 °C. (Ratomski & Hawrot-Pa 2021).Cells counting was performed daily, by microscope (Olympus IX81 fluorescence microscope), until the stationary phase was reached.

Nutrient Content in The Wastewater

Dairy wastewater contains several organic and inorganic components coming from the processing of the milk. Ammonia, total nitrogen, and phosphate content were evaluated before and after the cultivation of *C. vulgaris* as it directly affects not only the growth of the microalgae but also the quantitative and qualitative lipids content.

The nutrient removal in the media after algae cultivation was calculated using the following equation (Ratomski and Hawrot-Pa 2021):

$$W(\%) = \frac{C_I - C_N}{C_I} \times 100 \qquad (1)$$

where, CI and CN represent the average concentration of nutrients (mg/L) at the initial time and at time n, respectively

Determination of Algal Biomass

A double beam UV–Vis spectrophotometer (UV-1800, Shimadzu) was used to identify the microalgae cell growth by measuring the absorbance at 680 nm (Peter *et al.* 2021). The dry cell weight (DCW) was calculated as follows; 5 ml of aliquots of culture was filtered by a cellulose acetate membrane filter (0.45 μm pore size). Afterward, the filters were dried for 8 h at 90 ◦C and by the weight of the empty and full filter, the DCW was calculated. Then, by the absorbance values at 680 nm, the biomass concentration was obtained. A blank sample of each culture combination was used to reduce the interference of the medium color in the absorbance area. The filtrate was then centrifuged at 6000 rpm for 15 min; the supernatant was collected and the pellet was lyophilized and used for further qualitative and quantitative analysis.

The biomass growth productivity (PB), expressed as (mg/L/d) was calculated by the equation below (Goh *et al.* 2019):

$$PB = \frac{W_2 - W_1}{t_2 - t_1} \quad (2)$$

While the specific growth rate, μ (d^{-1}), as follows:

$$\mu = \frac{\ln(W_2 - W_1)}{t_2 - t_1} \quad (3)$$

where, W_1 and W_2 are the dry cell weight (mg/L) at time starting (t_1) and ending (t_2) time, respectively.

Extraction of Algal Oil from *C. vulgaris*

Algal oil was extracted following the sh method with minor modification (Davoodbasha *et al.* 2021). 5 mL of methanol and 5 mL of chloroform were added to 100 mg of dried and ground algal biomass. The biomass-solvents mixture was vigorously shaken for 30 minutes to favor the extraction. These steps were repeated twice to maximize the extraction yield. Methanol: chloroform: distilled water (1:2:1) mixture was then added and the mixture again vigorously shacken and then let to settle in a separating funnel. The solvent layer was separated by using anhydrous Na_2SO_4. The final product was collected and dried in a ventilated oven at 50°C until a constant weight was achieved. The lipid content was quantified gravimetrically.

Biodiesel Production and FAME Profile

The biodiesel was obtained by transesterification following the procedure reported in a previous work (Khan *et al.* 2023). In brief, 100 g of extracted algae oil was firstly heated at 120 °C for 15 min under stirring to remove the moisture and then transferred to a 250 mL glass flask. Afterward, a 50 ml of methanol solution containing 2g of the catalyst (KOH) was prepared. A defined volume of the methoxide solution was poured into the oil to have the alcohol: oil molar ratio of 12:1, and the reaction was left to run at 65 °C for 30 minutes.

Then, the solution transferred in a separating funnel and the two phases were left to separate by gravity. The biodiesel was separated from the glycerol and washed one time with an acetic acid solution (1% v/v) and three times with distilled water to remove side products, unreacted catalysts, and soaps. Then, the obtained biodiesel was heated at 150 °C for 30 min to remove the residual moisture coming from the washing process.

The biodiesel yield was calculated using the following equation (Roosta & Bardool 2019):

$$Y = \frac{M_b}{M_o} \quad (4)$$

where, Y represents the yield (g), Mb is the mass of biodiesel (g), and Mo is the mass of algae oil (g).

The qualitative and quantitative analysis of the obtained product was performed by gas chromatography (FID detector) using a well established methodology (*Khan et al. 2023*).

The sample was directly collected from the separation funnel and before being analyzed, the biodiesel was washed as reported above.

The following gas chromatography setup has been used: capillary column 30 × 0.31 × 0.25; helium as gas carrier, flow rate of 1 mL/min; temperature injection 280 °C; temperature detector 300°C; and temperature ramp 10 °C/min starting from 220 °C.

The total FAME (Fatty Acid Methyl Esters), expressed in percentage, was calculated referring to the total mass of the biodiesel using the following equation (Roosta & Bardool 2019):

$$FAME\ (\%) = \frac{\sum m}{b} \times 100 \quad (5)$$

where *m* is the mass of each fatty acid (g) and *b* is the total biodiesel mass (g).

The measurements were performed in triplicate and the obtained results compared to the EN 14214 standard.

Biodiesel Physical-Chemical Evaluation

1. Kinematic Viscosity

The kinematic viscosity represents one of the reasons why biodiesel is used as an alternative fuel instead of using directly vegetable oils or animal fats (Hossain & Davies 2010). The kinematic viscosity of the biodiesel, the petrol-diesel, and their blends has been measured following the EN ISO 3104 and EN ISO 3105 procedures. According to EN ISO 3104, 05, the kinematic viscosity must be in the range of 1.9 and 6.0 mm^2/s for the. The dynamic viscosity was determined using Anton-Paar MCR 502 at 40 °C as already described in the report in our previous work (Khan *et al.* 2023). Each measurement has been performed in triplicate at three shear rates: 7.5 s^{-1}; 15 s^{-1} and 37 s^{-1}.

By knowing the dynamic viscosity, the kinematic viscosity was obtained by the following equation:

$$v = \frac{\eta}{\rho} \qquad (6)$$

where, v, η, ρ, are the kinematic viscosity (m^2/s), the fluid density (kg/m^3), and the dynamic viscosity (Pa·s), respectively**.**

2. Calorific Value

The calorific value was measured by the oxygen bomb calorimeter following the ASTMD240-14 standard. The calorimeter was calibrated using benzoic acid at 20 °C (Verevkin *et al.* 2022).

3. Flash Point

The flash point of the biodiesel, fossil diesel, and their blends was measured with a Pensky–Martens closed-cup apparatus following the protocol reported by the EN 14214 standard. According to the EN standard, the minimum temperature is 101 °C.

4. Density

The density is defined as the mass per unit volume. In biodiesel, there is a reverse correlation between the molecular weight of the fatty acid chains and the amount of unsaturation. In other words, the density increases when the molecular weight and the unsaturation decrease (Giakoumis 2013). Generally, biodiesel fuels have a higher density than fossil diesel and produce more than three times the energy of the same amount of fossil fuel (Tesfa *et al.* 2010).

The biodiesel density was measured following the EN ISO (International Organization for Standardization) 12185 test method. The EN 14214 sets the density at 15 °C at between 860-900 kg/m^3.

5. Cetane Number

The cetane number (CN) represents the ignitability of the fuel. The CN is a fundamental property of biodiesel and is critical during engine start, especially in cold conditions. CN is defined as the percentage by volume of the normal cetane in a mixture of normal cetane and α-methyl naphthalene that has the same ignition characteristics (ignition delay) as the test fuel when combustion is carried out in a standard engine under specified operating conditions (Hossain & Davies 2010).The CN requirement for the engine depends on the composition of the fuel, which is related to the feedstock (Demirbas 2007). CN decreases as unsaturation increases. A low cetane number leads to a long ignition delay; conversely, biodiesel containing low unsaturated fatty acids has a higher CN, meaning a fast and smooth engine operation (Nabi *et al.* 2009; Chalatlon *et al.* 2011). Compared to fossil diesel, the CN in biodiesel is generally higher.

6. Elemental Analysis

The biodiesel and diesel content in carbon, hydrogen, nitrogen, sulfur, and oxygen has been measured by Thermo Scientific Flash 2000 CHNS/O.

Generator Performance Test

To evaluate the engine performance using the biodiesel-made oil extracted by *C. vulgaris* cultivated in dairy wastewater, alone and blended with the fossil diesel, the electric generator tests were performed.

The generator was fueled with pure biodiesel (100%) and with biodiesel-diesel blends at 10, 25, 50, and 75% biodiesel.

The electric generator (Denyo DA-2805) connected to an electric dynamometer operated at 100%, 75%, 50%, 25%, and 0% of loading and 3,000 rpm.

At each load percentage, the following were measured; i) the engine speed; ii) the fuel flow rate; and iii) the brake torque. The concentration of CO, CO_2, and NO_x emitted were also monitored by an emission analyzer (Testo model 350XL), and the smoke concentration by a smoke meter (HORIBA model MEXA-130S).

The brake-specific fuel consumption (BSFC) was calculated as follows (Emaish *et al.* 2021)

$$BSFC = \frac{F_c}{P_b} \quad (7)$$

where, F_c is the fuel mass flow rate (kg/s) and P_b the brake power output (kW).

Statistical Analysis

All the experiments were performed in triplicate and the average values with standard deviation (SD) are reported.

3. RESULTS AND DISCUSSION

Effect Of Wastewater Dilution on *C. vulgaris* Biomass

In Table 1 the physicochemical characteristics of the wastewater used for growing algae are reported (mg/L).

Table 1. Chemical characteristics of the wastewater used for algae growing. Values are expressed in mg/L

Parameters	Values
NH_3	52
NO_3	69
PO_4	600
pH	8

The influence of dairy wastewater dilution (10, 25, 50, and 75% v/v in distilled water) on the biomass production of *Chlorella vulgaris* is presented in Figure 2A. Algal growth in wastewater-based media was compared with that in the control BG11 medium. Overall, a non-linear response was observed, with maximum biomass productivity recorded at 50% dilution (3.59 g L^{-1}), followed by 25% dilution (3.13 g L^{-1}). The control medium yielded 2.05 g L^{-1}, whereas significantly lower biomass concentrations were obtained at 75% (0.78 g L^{-1}) and 10% (0.57 g L^{-1}) dilutions. A corresponding effect on growth kinetics was also observed. At the two extreme dilutions (10% and 75%), cultures reached the stationary phase after approximately 10 days. In contrast, cultures grown at 25% and 50% dilution exhibited prolonged exponential growth and entered the stationary phase only after 12 days. For comparison, *C. vulgaris* grown in BG11 medium typically requires 14–16 days to reach the stationary phase under similar conditions (Chew *et al.* 2018), indicating that wastewater composition significantly modifies growth dynamics.

The observed biomass trend can be attributed to the balance between nutrient availability and environmental stressors. At intermediate dilutions (25–50%), dairy wastewater provides sufficient concentrations of bioavailable nitrogen and phosphorus to support rapid cell division and sustained metabolic activity. In contrast, higher dilution (10%) results in nutrient limitation, restricting protein synthesis and photosynthetic efficiency, which in turn suppresses biomass accumulation. Conversely, at higher wastewater concentrations (75%), despite increased nutrient availability, biomass production was reduced. This effect is likely associated with inhibitory factors such as increased turbidity and colour intensity of dairy effluent, which reduce light penetration and limit photosynthetically active radiation reaching the cells. Since light availability is a key determinant of microalgal productivity, reduced irradiance can significantly impair growth even under nutrient-replete conditions (Khalaji *et al.* 2021). In addition, potential accumulation of organic and inorganic inhibitory compounds in undiluted wastewater may further contribute to growth suppression. The control medium, while chemically defined and transparent, supported lower biomass compared to optimal wastewater dilutions, highlighting the beneficial role of wastewater-derived nutrients. Continuous 24 h illumination using LED light sources on both sides of the photobioreactor minimized diel light limitation and ensured consistent photosynthetic activity across conditions.

These findings underline that both nutrient concentration and optical properties of the cultivation medium play critical roles in determining *C. vulgaris* growth performance, and that an optimal wastewater dilution is essential to balance nutrient supply with light availability.

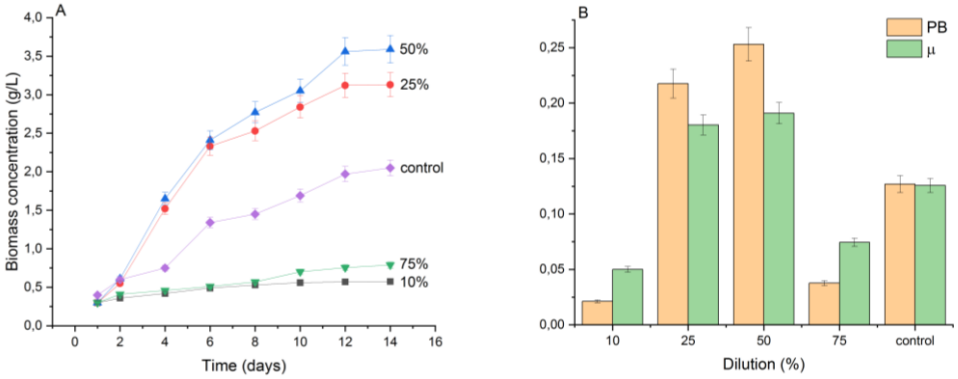


Figure 2. A) Biomass concentration expressed in g/L versus time (days) at different wastewater dilutions. Control represents water as a growing medium. The data are expressed as the average with the respective SD (n=3); B) biomass growth productivity (PB) and biomass growth rate (μ) at different wastewater dilutions (v/v in %). The results refer to the 14th day of cultivation. The average values are reported with the SD (n=3).

Table 2. Dry cell weight content (DCW), biomass productivity (Pb), and specific growth rate (μ) of *C. vulgaris* grown in control media and dairy wastewater and various dilutions. Support of Figure 2B

Growing Media	DCW (g/L)	Pb (mg/L/d)	μ(d^{-1})
Control	2.05 ± 0.08	0.12 ± 0.01	0.12 ± 0.02
10%	0.57 ± 0.01	0.02 ± 0.01	0.05 ± 0.01
25%	3.13 ± 0.02	0.21 ± 0.03	0.18 ± 0.03
50%	3.59 ± 0.06	0.25 ± 0.01	0.19 ± 0.03
75%	0.78 ± 0.02	0.03 ± 0.01	0.07 ± 0.01

In Table 2, the calculated parameters including daily biomass productivity and basic growth rate of *C. vulgaris* on the 14th day are resumed. Although the medium with lower dilution was the lightest in color intensity after the control, the biomass productivity measured was almost 6 times lower than in the control.

As reported in a similar work (Peter *et al.* 2021).the reduction in biomass growth could be ascribed to the lack of nutrient supply in the culture medium. For an optimal cultivation process, a proper mix of nutrient sources with adequate light penetration must be ensured. Thus, it is not desirable to entirely substitute the inorganic medium (Machado *et al.* 2020). Other influencing factors such as temperature, and CO_2 supply were kept constant during the cultivation process to avoid any additional influences on biomass development *(*Eloka-Eboka & Inambao 2017).

Effect of Different Dilutions of Wastewater on Nutrient Removal

The data in Table 3 shows a difference between nitrates, phosphate, and ammonia biological removal at different wastewater dilutions. The higher values of nitrate and ammonia removal are observed at 75% dilution, while phosphate is at 50%. The lowest values for all the parameters are at 10% dilution.

Table 3 Removal of nitrate, phosphate, and ammonia from wastewater at different dilutions by the microalgae after the 14th day of growing

Parameters	Wastewater Dilution (v/v)			
	10%	25%	50%	75%
NO_3	17.14 ± 0.03	19.23 ± 0.01	29.16 ± 0.05	38.34 ± 0.01
PO_4	19.87 ± 0.01	23.44 ± 0.01	39.72 ± 0.01	39.07 ± 0.01
NH_3	19.53 ± 0.01	36.18 ± 0.01	41.94 ± 0.01	43.61 ± 0.01

The reason for efficient performance in wastewater and effluent of the dairy industry may be the presence of enough nutrients, including nitrogen and phosphorus, essential microalgae growth. Previous works (Kothari *et al.* 2013; Khalaji *et al.* 2021) have shown that algae need NH_3-N and phosphorus to produce amino acids and phospholipids respectively (Barsanti & Gualtieri 2014). In another study dealing with the isolation of *Chlorella vulgaris* from pig wastewater and its ability to remove nutrients from untreated wastewater, it has been reported that total nitrogen removal after 12 days is 90% which concerns the ability of *C. vulgaris* to absorb nitrogen; this study is consistent with the present study.

Lipid Extraction from *Chlorella vulgaris* Biomass

Chlorella vulgaris was identified as the dominant microalgal species in the sampled environment, exhibiting enhanced biomass productivity and lipid accumulation potential (Ratomski & Hawrot-Paw 2021; Khalaji *et al.* 2021; Zhang *et al.* 2014). The total lipid content of the cultivated biomass was 24 ± 2%, with variation dependent on the cell disruption strategy employed (data not shown). This value exceeds that reported previously for comparable systems, where a lipid content of 18.7 wt.% was obtained (dos Santos *et al.* 2015), indicating improved lipid accumulation under the present conditions.

Lipid recovery from microalgae is strongly governed by the efficiency of cell disruption, which facilitates the release of intracellular lipids through the breakdown of rigid cell wall structures into the surrounding medium (Kumar *et al.* 2014; Couto *et al.* 2022). In this study, a mechanical grinding approach was employed to enhance cell disruption and maximise lipid liberation. Following grinding, a lipid yield of 3 mg L^{-1} was obtained for *C. vulgaris*. These results are consistent with previous reports (Nguyen *et al.* 2019) and further demonstrate the robustness and scalability potential of the applied disruption method for downstream lipid recovery processes.

Physical-Chemical Characterization

1. Biodiesel Yield, Kinematic Viscosity, Density, and Calorific Value

In the Table 4 the physical-chemical properties of biodiesel obtained by *C. vulgaris* compared to diesel and the EN and ASTM values are illustrated.

Table 4. Physical-chemical properties of biodiesel obtained by *C. vulgaris* compared to diesel and the EN and ASTM values

	Biodiesel from *C. vulgaris*	**Diesel**	**EN14214**	**ASTM D61**
Yield (g/100g oil)	93 ± 4			
K.Viscosity (40 °C mm^2/s^2)				
7.5 s^{-1}	4.9 ± 0.3	2.6	3.5-5.0	1.9-6.0
15 s^{-1}	4.3 ± 0.1			
37 s^{-1}	4.1 ± 0.4			
Calorific value (MJ/kg)	37.5 ± 1.4	42.2		
Density (15 °C, g/cm^3)	0.875 ± 0.019	0.850	0.860-0.900	0.880
Flashpoint (°C)	131 ± 2	73	101 min	130 min
Cetane number	50.21 ± 0.13 (36.19 ± 0.13)*	56.66 ± 0.19		
Carbon	81.1	87.1		
Hydrogen	10.6	13.6		
Oxygen	7.5			
Nitrogen	0.8			
C/H	7.6	6.4		

*cetane number of oil extracted from *C. vulgaris*

A previous study reported that optimal transesterification conditions using methanol and KOH can significantly enhance biodiesel yield (Khan *et al.* 2023). However, that work was based on alternative lipid feedstocks, namely sunflower and rapeseed oils. The optimized reaction parameters included a methanol-to-oil molar ratio of 12:1, KOH as catalyst (40 mg mL^{-1} methanol), and reaction conditions of 65 °C for 30 min.

In the present study, applying identical reaction conditions yielded a biodiesel conversion efficiency of 93 ± 4%, which is marginally lower than that reported for rapeseed and sunflower oil under comparable conditions. Nevertheless, when benchmarked against studies employing *Chlorella vulgaris* as feedstock (Asadi *et al.* 2020; Scarponi *et al.* 2021; Katırcıoğlu Sınmaz *et al.* 2023), the obtained yield remains highly competitive and exceeds the commonly reported threshold of 90 g per 100 g of oil (Table 4), indicating efficient conversion under the applied protocol.

From an application perspective, fuel viscosity is a critical parameter in biodiesel utilization, as it directly influences fuel atomization, engine startability, and overall combustion performance. In particular, kinematic viscosity at 40 °C is a key specification defined in biodiesel and petroleum diesel standards. As previously reported for KOH-catalysed transesterification systems, viscosity is strongly dependent on reaction conditions, particularly the alcohol type and the alcohol-to-oil molar ratio (Sharma *et al.* 2020).

In this study, kinematic viscosity at 40 °C, determined according to EN standard methods, ranged from 3.6 to 5.2 $mm^2\ s^{-1}$, showing an inverse relationship with shear rate. Specifically, higher shear rates resulted in lower viscosity values, consistent with shear-thinning behaviour during measurement. However, viscosity values across all shear rates were statistically comparable, indicating no significant differences between conditions.

Together with the kinematic viscosity, the biodiesel density provides information to predict the engine power performance and consequently the fuel consumption (Aydın 2020). The biodiesel's density is related to the purity of the feedstock, the fatty acids composition, and the total content. The density at 15°C was 0.875 ± 0.019 and it represents an acceptable value as it falls in the required range of 0.860 – 0.900 gm/cm^3

The calorific value is the thermal energy released by the combustion of a unit of fuel, giving the energy content of the fuel (Dey *et al.* 2021). As for the viscosity, the calorific value depends on the nature of the feedstock and only in minor by the transesterification conditions. Using *C. vulgaris* as an oil source, the calorific value of the produced biodiesel was 37.5 ± 1.4 MJ/Kg, which is a positive value in line with the EN 14214 and ASTM D6751 requirements.

2. Flash Point

The flash point is the lowest temperature at which a sufficient concentration of vapors is released from the fuel, which upon mixing with air ignite (Santos *et al.* 2020). The presence of fatty acid methyl esters is responsible for the higher biodiesel flashpoint compared to the fossil diesel making it safer to handle at high temperatures. As biodiesel, in the automotive, is used in a mixture with fossil diesel, the flashpoint of pure biodiesel and blends of biodiesel-diesel has been evaluated and the results are reported in Figure 3.

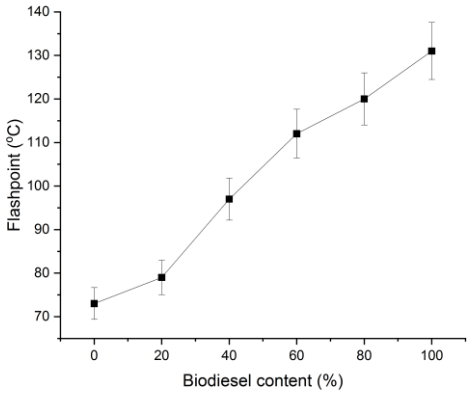


Figure 3. Correlation between flash point (°C) and biodiesel content in the biodiesel-diesel mixture. 0% diesel, 100% pure biodiesel. The average value and the S.D. are reported (n=3).

The flash point of pure biodiesel varies according to the source and the quality of the oil; herein the flash point temperature obtained was 131°C, almost double that of the fossil diesel 73°C. As already reported (Khan *et al.* 2023), in biodiesel-diesel blends, by increasing the content of biodiesel, the flash point decreases in a quasi-linear correlation. The clear correlation between the biodiesel quantity in a diesel-biodiesel blend and its flash point. This means that the experimental determination of a flash point temperature also provides information regarding the biodiesel content of diesel-biodiesel blends. Conversely, if the percentage of biodiesel in a diesel-biodiesel blend is known, the flashpoint can be estimated if the flashpoints of diesel and pure biodiesel are known. From the obtained results, it is also clear that, for diesel-biodiesel blends with lower contents of biodiesel, the flash point is mainly determined by that of the mineral diesel.

3. FAME composition

The lipids extracted from *C. vulgaris* were 24% of the dried weight (30 g of the algal dried biomass). The content, obtained by gravimetric analysis, makes the selected biomass and its growing approach a suitable strategy for the 3rd generation type biodiesel production.

It is well established that the quality of the biodiesel is determined by the fatty acid content, in particular their molecular weight and the saturation and unsaturation degree. Biodiesels containing saturated and mono-unsaturated fatty acids result in superior quality as reported by international standards. The FAME (Fatty Acid Methyl Ester) profile of the obtained biodiesel from *C. vulgaris* biomass is resumed in Table 5. The data shows a major content of saturated and mono-unsaturated FAME. The mains are Tridecanoic acid (C13:0), Palmitic acid (C16:0), Erucic acid (22:1) Myristoleic acid (C14:1), Linolelaidic acid (C18:2), G Linolenic acid (C18:3). The obtained results indicate that the biodiesel obtained from *Chlorella vulgaris* cultivated in dairy wastewater has 33.53% saturated, 28.68% monounsaturated and 37.12% polyunsaturated fatty acids content (Davoodbasha *et al.* 2021).

Table 5. Fatty Acid Methyl Ester (FAME) present in biodiesel from *C. vulgaris* cultivated in dairy wastewater. The content is expressed in percentages referring to the total amount of FAME.

FAME (Fatty Acid Methyl Ester)	(%)
Tridecanoic acid (13:0)	4.02
Myristoleic acid (14:1)	7.95
Palmitic acid (16:0)	52.4
Stearic acid	17.3
Linolelaidic acid (18:2)	6.83
Linoleic acid (18:3)	7.90
Oleic acid	5.21
cis, 11, 14-Eicosadienoic acid (20:2)	4.70
Arachidonic acid(20:4)	3.09
Erucic acid (22:1)	4.46
Tricosanoid acid (23:0)	3.33

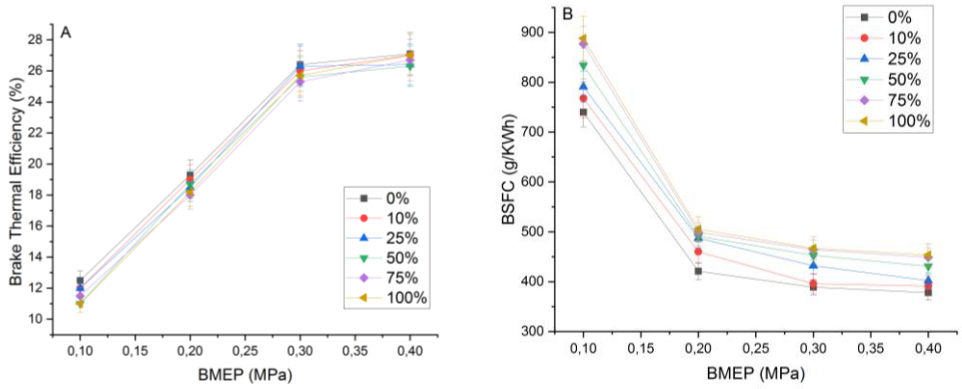


Figure 4. A) brake thermal efficiency (%) and B) brake specific fuel consumption VS BMEP in various biodiesel-diesel blends. 0%= pure diesel; 100% = pure biodiesel. Average values are reported with SD (n=3)

As shown in Figure 4A, the brake thermal efficiency increases consistently with increasing BMEP for all tested fuel blends. At low engine load (0.10 MPa), BTE values are relatively low (≈11–13%), which can be attributed to incomplete combustion and higher relative heat losses. As the load increases to 0.30–0.40 MPa, BTE rises significantly, reaching maximum values of approximately 26–27%. This trend is widely reported in compression ignition engines and is associated with improved combustion efficiency at higher loads, where a larger fraction of the fuel energy is effectively converted into useful work (Rakopoulos *et al.* 2006; Lapuerta *et al.* 2008). A slight variation in BTE can be observed among the different blends. Higher blend ratios tend to exhibit marginally lower BTE compared to the baseline fuel (0%), particularly at higher loads. This behavior is commonly attributed to the lower calorific value and higher viscosity of biodiesel fuels, which can impair fuel atomization and air–fuel mixing, leading to less efficient combustion (Knothe 2010; Xue *et al.* 2011). Nevertheless, the relatively small differences observed indicate that the blends maintain comparable thermal efficiency to conventional diesel under most operating conditions, consistent with previous studies on biodiesel–diesel blends (Lapuerta *et al.* 2008).

Figure 4B shows the variation of BSFC with BMEP, exhibiting an inverse trend compared to BTE. BSFC decreases sharply with increasing engine load for all tested fuels. At low BMEP (0.10 MPa), BSFC values are significantly higher (≈750–900 g/kWh), reflecting inefficient fuel utilization due to incomplete combustion, higher heat losses, and a greater proportion of frictional power relative to brake power (Rakopoulos *et al.* 2006). As the load increases, BSFC decreases substantially, reaching minimum values in the range of 380–470 g/kWh at 0.30–0.40 MPa. This improvement is attributed to enhanced combustion efficiency and better utilization of the supplied fuel energy at higher loads (Xue *et al.* 2011).

Regarding the effect of fuel blends, higher biodiesel proportions generally lead to increased BSFC across all operating conditions. This is primarily due to the lower heating value of biodiesel compared to petroleum diesel, requiring a greater mass of fuel to generate the same power output (Knothe 2010). Additionally, differences in fuel properties such as density and viscosity may influence injection characteristics and combustion behavior (Xue *et al.* 2011). The increase in BSFC is more pronounced at lower loads, where combustion inefficiencies are already significant. At higher loads, however, the differences between blends become less distinct, suggesting improved combustion of biodiesel blends under these conditions, likely due to their inherent oxygen content, which promotes more complete combustion (Lapuerta *et al.* 2008).

Overall, the combined analysis of BTE and BSFC indicates that although biodiesel blends—such as those potentially derived from microalgal sources—may slightly reduce engine efficiency and increase fuel consumption, their performance remains comparable to that of conventional diesel, particularly at medium to high engine loads. These findings are consistent with the broader literature and support the feasibility of biodiesel as a sustainable alternative fuel without requiring significant engine modifications (Xue *et al.* 2011).

Figure 5 presents correlations between biodiesel concentration and the variation in emissions. As increasing biodiesel concentration, CO and smoke emissions decreased, while NO_x and CO_2 increased. The percent change in emissions of CO_2, NO_x, CO, and smoke, using pure-form biodiesel, was +18%, +15%, -60%, and -19%, respectively. These results were attributed to the improvement of combustibility and the increase in combustion temperature due to oxygen-containing biodiesel.

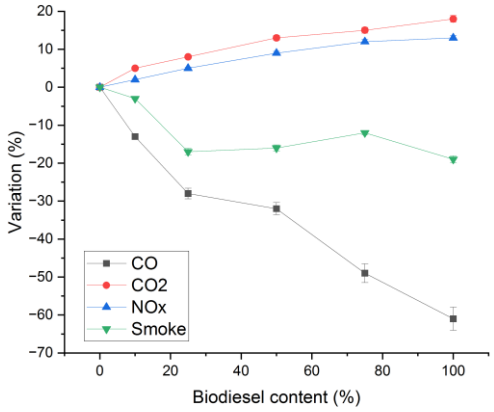


Figure 5. Variation of CO, CO_2, NO_x, and smoke emissions in different blends of diesel-biodiesel. The variation is expressed as a percentage in comparison to the emission from diesel. (0%= pure diesel considered as zero point; 100% = pure biodiesel). Average values are reported with SD (n=3)

4. CONLUSION

This study demonstrates that dairy wastewater represents a promising cultivation medium for *Chlorella vulgaris*, enabling enhanced lipid production for biodiesel applications. The nutrient-rich composition of dairy effluent, particularly its elevated nitrogen and phosphorus content, supports robust algal growth while simultaneously enabling the valorisation of a nutrient-laden waste stream. Compared with conventional growth media, cultivation of *C. vulgaris* in dairy wastewater resulted in increased lipid accumulation (33 % saturated, 28 % monounsaturated and 37 % polyunsaturated), highlighting the potential of wastewater-based cultivation strategies to enhance lipid biosynthesis. In addition, this integrated approach couples biomass production with wastewater treatment, thereby contributing to the mitigation of environmental impacts associated with dairy effluents.Despite these promising outcomes, several limitations should be acknowledged. The study was conducted under controlled laboratory conditions, which may not fully replicate the variability and complexity of real wastewater systems at industrial scale. Furthermore, only a limited set of operational parameters and a single algal strain were evaluated, restricting broader generalisation. Additional constraints include the lack of long-term cultivation stability assessment and limited techno-economic analysis, which are essential for evaluating large-scale feasibility.Overall, the dual role of dairy wastewater as both a growth substrate and a remediation agent underscores its potential as a sustainable feedstock for microalgae-based biodiesel production. Future work should focus on process optimisation, scale-up validation, and integrated life-cycle and economic assessments to fully establish its viability within a circular bioenergy framework.

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AUTHOR'S DECLARATION

The author's declare that i) there are no conflicts of interests; ii) all the Figures and Tables in the manuscript are ours; iii) no animal or human studies are present in the manuscript and iv) no potentially identified images or data are present in the manuscript.

REFERENCES

Abreu, A.P. (2012). Mixotrophic cultivation of *Chlorella vulgaris* using dairy waste. *Bioresource Technology*, 108, 60–66.

Asadi, P., Rad, H. A., & Qaderi, F. (2020). Lipid and biodiesel production by cultivation isolated strain Chlorella sorokiniana pa. 91 and Chlorella vulgaris in dairy wastewater treatment plant effluents. *Journal of Environmental Health Science and Engineering*, *18*, 573-585. https://doi.org/10.1007/s40201-020-00483-y.

Aydın, S. (2020). Comprehensive analysis of combustion, performance and emissions of power generator diesel engine fueled with different source of biodiesel blends. *Energy*, 205, 118074. https://doi.org/10.1016/j.energy.2020.118074.

Barman, D. K., Islam, R., Dash, G. K., Ganguli, S., Chakraborty, A. K., Kabir, M., & Sabur, M. A. (2024). Interfering effect of silicate in the quantification of phosphate by the molybdenum-blue method. *Asian Journal of Water, Environment and Pollution*, *21*(6), 195-201. https://doi.org/10.3233/ajw240087.

Barsanti, L., & Gualtieri, P. (2014). Algae: anatomy, biochemistry, and biotechnology. CRC press, p 295.

Brennan, L., & Owende, P. (2010). Biofuels from microalgae—areview of technologies for production, processing, and extractions of biofuels and co-products. *Renew Sust Energ Rev., 14*, 557–577. https://doi.org/10.1016/j.rser.2009.10.009.

Chalatlon, V., Roy, M. M., Dutta, A., & Kumar, S. (2011). Jatropha oil production and an experimental investigation of its use as an alternative fuel in a DI diesel engine. *Journal of Petroleum Technology and Alternative Fuels*, *2*(5), 76-85.

Chen, J., Li, J., Dong, W., Zhang, X., Tyagi, R. D., Drogui, P., & Su-rampalli, R. Y. (2018). The potential of microalgae in biodiesel production. *Renewable and Sustainable Energy Reviews, 90*, 336-346.

Chew, K. W., Chia, S. R., Show, P. L., Ling, T. C., Arya, S. S., & Chang, J. S. (2018). Food waste compost as an organic nutrient source for the cultivation of *Chlorella vulgaris*. *Bioresource technology*, *267*, 356-362. https://doi.org/10.1016/j.biortech.2018.07.069.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnol Adv*., *25*, 294–306.

Choudhury, P., Ray, R.N., Bandyopadhyay, T.K., Basak, B., Muthuraj, M., & Bhunia, B. (2021). Process engineering for stable power recovery from dairy wastewater using microbial fuel cell. *Int. J. Hydrogen Energy, 46*, 3171–3182. https://doi.org/10.1016/j.ijhydene.2020.06.152.

Couto, D., Melo, T., Conde, T. A., Moreira, A. S., Ferreira, P., Costa, M., & Domingues, P. (2022). Food grade extraction of Chlorella vulgaris polar lipids: A comparative lipidomic study. *Food Chemistry*, *375*, 131685. https://doi.org/10.1016/j.foodchem.2021.131685.

Davoodbasha, M., Pugazhendhi, A., Kim, J. W., Lee, S. Y., & Nooruddin, T. (2021). Biodiesel production through transesterification of Chlorella vulgaris: Synthesis and characterization of CaO nanocatalyst. *Fuel*, *300*, 121018. https://doi.org/10.1016/j.fuel.2021.121018.

Demirbas, A. (2007). Progress and recent trends in biofuels. *Progress in Energy and Combustion Science*, *33*(1), 1-18. https://doi.org/10.1016/j.pecs.2006.06.001.

Dey, P., Ray, S., & Newar, A. (2021). Defining a waste vegetable oil-biodiesel based diesel substitute blend fuel by response surface optimization of density and calorific value. *Fuel, 283*, 118978. https://doi.org/10.1016/j.fuel.2020.118978.

Dongre, A., Sogani, M., Sonu, K., Syed, Z., & Sharma, G. (2020). Treatment of dairy wastewaters: evaluating microbial fuel cell tools and mechanism. In Environmental Issues and Sustainable Development, IntechOpen: Rijeka, Croatia. https://doi.org/10.5772/intechopen.93911.

dos Santos, R. R., Moreira, D. M., Kunigami, C. N., Aranda, D. A. G., & Teixeira, C. M. L. L. (2015). Comparison between several methods of total lipid extraction from Chlorella vulgaris biomass. *Ultrasonics sonochemistry*, *22*, 95-99. https://doi.org/10.1016/j.ultsonch.2014.05.015.

Eloka-Eboka, A. C., & Inambao, F. L. (2017). Effects of CO2 sequestration on lipid and biomass productivity in microalgal biomass production. *Applied Energy*, *195*, 1100-1111. https://doi.org/10.1016/j.apenergy.2017.03.071.

Emaish, H., Abualnaja, K. M., Kandil, E. E., & Abdelsalam, N. R. (2021). Evaluation of the performance and gas emissions of a tractor diesel engine using blended fuel diesel and biodiesel to determine the best loading stages. *Scientific Reports*, *11*(1), 9811. https://doi.org/10.1038/s41598-021-89287-0.

Galasso, C., Gentile, A., Orefice, I., Ianora, A., Bruno, A., Noonan, D.M., Sansone, C., Albini, A., & Brunet, C., (2019). Microalgal derivatives as potential nutraceutical and food supplements for human health: A focus on cancer prevention. *Nutrients, 11*(6), 1226. https://doi.org/10.3390/nu11061226.

Giakoumis, E. G. (2013). A statistical investigation of biodiesel physical and chemical properties, and their correlation with the degree of unsaturation. *Renewable Energy*, *50*, 858-878. https://doi.org/10.1016/j.renene.2012.07.040.

Goh, B. H. H., Ong, H. C., Cheah, M. Y., Chen, W. H., Yu, K. L., & Mahlia, T. M. I. (2019). Sustainability of direct biodiesel synthesis from microalgae biomass: A critical review. *Renewable and Sustainable Energy Reviews*, *107*, 59-74. https://doi.org/10.1016/j.rser.2019.02.012.

Gouveia, L., Batista, A. P., Miranda, A., Empis, J., & Raymundo, A. (2007). Chlorella vulgaris biomass used as colouring source in traditional butter cookies. *Innovative Food Science & Emerging Technologies, 8*(3), 433-436. https://doi.org/10.1016/j.ifset.2007.03.026.

Gramegna, G., Scortica, A., Scafati, V., Ferella, F., Gurrieri, L., Giovannoni, M., & Benedetti, M. (2020). Exploring the potential of microalgae in the recycling of dairy wastes. *Bioresource Technology Reports*, 12, 100604. https://doi.org/10.1016/j.biteb.2020.100604.

Hossain, A. K., & Davies, P. A. (2010). Plant oils as fuels for compression ignition engines: A technical review and life-cycle analysis. *Renewable energy*, *35*(1), 1-13. https://doi.org/10.1016/j.renene.2009.05.009.

Hosseini Tafreshi, A., & Shariati, M. (2009). Dunaliella biotechnology: methods and applications. *J Appl Microbiol, 107*, 14–35. https://doi.org/10.1111/j.1365-2672.2009.04153.x.

Hu, J., Zhuang, Z., Pan, M., Han, T., Li, Y., & Liu, W. (2022). A method of the determination nitrate nitrogen (NO3−-N) in high-salinity water samples. *Chemical Papers*, *76*(3), 1739-1744. https://doi.org/10.1007/s11696-021-02007-w.

Katırcıoğlu Sınmaz, G., Erden, B., & Şengil, I. A. (2023). Cultivation of Chlorella vulgaris in alkaline condition for biodiesel feedstock after biological treatment of poultry slaughterhouse wastewater. *International Journal of Environmental Science and Technology*, *20*(3), 3237-3246. https://doi.org/10.1007/s13762-022-04137-4.

Khalaji, M., Hosseini, S. A., Ghorbani, R., Agh, N., Rezaei, H., Kornaros, M., & Koutra, E. (2021). Treatment of dairy wastewater by microalgae Chlorella vulgaris for biofuels production. *Biomass Conv. Bioref, 13*, 3259–3265. https://doi.org/10.1007/s13399-021-01287-2.

Khan, E., Ozaltin, K., Spagnuolo, D., Bernal-Ballen, A., Piskunov, M. V., & Di Martino, A. (2023). Biodiesel from rapeseed and sunflower oil: effect of the transesterification conditions and oxidation stability. *Energies*, *16*(2), 657. https://doi.org/10.3390/en16020657.

Knothe, G. (2010). Biodiesel and renewable diesel: A comparison. *Progress in Energy and Combustion Science*, *36*, 364–373. https://doi.org/10.1016/j.pecs.2009.11.004.

Kothari, R., Prasad, R., Kumar, V., & Singh, D. (2013). Production of biodiesel from microalgae Chlamydomonas polypyrenoideum grown on dairy industry wastewater. *Bioresour Technol, 144*, 499– 503. https://doi.org/10.1016/j.biortech.2013.06.116.

Kumar, R. R., Rao, P. H., & Arumugam, M. (2014). Lipid extraction methods from microalgae: a comprehensive review. *Front Energy Res, 2*, 61–69. https://doi.org/10.3389/fenrg.2014.00061.

Lapuerta, M., Armas, O., & Rodríguez-Fernández, J. (2008). Effect of biodiesel fuels on diesel engine emissions. *Progress in Energy and Combustion Science*, *34*, 198–223. https://doi.org/10.1016/j.pecs.2007.07.001.

Machado, A., Pereira, H., Costa, M., Santos, T., Carvalho, B., Soares, M., ... & Silva, J. (2020). Development of an organic culture medium for autotrophic production of Chlorella vulgaris biomass. *Applied Sciences*, *10*(6), 2156. https://doi.org/10.3390/app10062156.

Martins, C. F., Pestana, J. M., Alfaia, C. M., Costa, M., Ribeiro, D. M., Coelho, D., & Prates, J. A. (2021). Effects of Chlorella vul-garis as a feed ingredient on the quality and nutritional value of weaned piglets' meat. *Foods, 10*(6), 1155. https://doi.org/10.3390/foods10061155.

Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: a review. *Renewable And Sustainable Energy Reviews, 14*(1), 217-232. https://doi.org/10.1016/j.rser.2009.07.020.

Nabi, M. N., Rahman, M. M., & Akhter, M. S. (2009). Biodiesel from cotton seed oil and its effect on engine performance and exhaust emissions. *Applied thermal engineering*, *29*(11-12), 2265-2270. https://doi.org/10.1016/j.applthermaleng.2008.11.009.

Nguyen, T. D. P., Nguyen, D. H., Lim, J. W., Chang, C. K., Leong, H. Y., Tran, T. N. T., ... & Show, P. L. (2019). Investigation of the relationship between bacteria growth and lipid production cultivating of microalgae Chlorella vulgaris in seafood wastewater. *Energies*, *12*(12), 2282. https://doi.org/10.3390/en12122282.

Peter, A. P., Chew, K. W., Koyande, A. K., Yuk-Heng, S., Ting, H. Y., Rajendran, S., ... & Show, P. L. (2021). Cultivation of Chlorella vulgaris on dairy waste using vision imaging for biomass growth monitoring. *Bioresource technology*, *341*, 125892. https://doi.org/10.1016/j.biortech.2021.125892.

Raghunath, B. V., Punnagaiarasi, A., Rajarajan, G., Irshad, A., Elango, A., & Mahesh Kumar, G. (2016). Impact of dairy effluent on environment—a review. Integrated Waste Management in India: Status and Future Prospects for Environmental Sustainability, 239-249. https://doi.org/10.1007/978-3-319-27228-3_22.

Rakopoulos, C. D., Antonopoulos, K. A., Rakopoulos, D. C., Hountalas, D. T., & Giakoumis, E. G. (2006). Comparative performance and emissions study of a direct injection diesel engine using blends of diesel fuel with vegetable oils or bio-diesels of various origins. *Energy conversion and management*, *47*(18-19), 3272-3287. https://doi.org/10.1016/j.enconman.2006.01.006.

Ratomski, P., & Hawrot-Paw, M. (2021). Influence of nutrient-stress conditions on *Chlorella vulgaris* biomass production and lipid content. *Catalysts, 11*(5), 573. https://doi.org/10.3390/catal11050573.

Lee, R. A., & Lavoie, J-M. (2013). From first- to third-generation biofuels: Challenges of producing a commodity from a biomass of increasing complexity. Animal Frontiers, 3(2), 6–11. https://doi.org/10.2527/af.2013-0010.

Roosta, A., & Bardool, R. (2019). A predictive correlation for dynamic viscosity of fatty acid methyl esters and biodiesel. *Journal of the American Oil Chemists' Society*, *96*(7), 741-750. https://doi.org/10.1002/aocs.12243.

Santos, S. M., Nascimento, D. C., Costa, M. C., Neto, A. M., & Fregolente, L. V. (2020). Flash point prediction: Reviewing empirical models for hydrocarbons, petroleum fraction, biodiesel, and blends. *Fuel, 263*, 116375. https://doi.org/10.1016/j.fuel.2019.116375.

Scarponi, P., Izzo, F. C., Bravi, M., & Cavinato, C. (2021). *C. vulgaris* growth batch tests using winery waste digestate as promising raw material for biodiesel and stearin production. *Waste Management*, *136*, 266-272. https://doi.org/10.1016/j.wasman.2021.10.014.

Sharma, A., Kodgire, P., & Kachhwaha, S. S. (2020). Investigation of ultrasound-assisted KOH and CaO catalyzed transesterification for biodiesel production from waste cotton-seed cooking oil: Process optimization and conversion rate evaluation. *Journal of Cleaner Production*, *259*, 120982. https://doi.org/10.1016/j.jclepro.2020.120982.

Silva, C. K. D., Almeida, A. C. A. D., Costa, J. A. V., & Morais, M. G. D. (2018). Cyanobacterial biomass by reuse of wastewater-containing hypochlorite. *Ind Biotechnol, 14*, 265–269. https://doi.org/10.1089/ind.2018.0014.

Tao, R., Kinnunen, V., Praveenkumar, R., Lakaniemi, A-M., & Rintala, J. A. (2017). Comparison of Scenedesmus acuminatus and Chlorella vulgaris cultivation in liquid digestates from anaerobic digestion of pulp and paper industry and municipal wastewater treatment sludge. *J Appl Phycol 29*, 2845–2856. https://doi.org/10.1007/s10811-017-1175-6.

Tesfa, B., Mishra, R., Gu, F., & Powles, N. (2010). Prediction models for density and viscosity of biodiesel and their effects on fuel supply system in CI engines. *Renewable energy*, *35*(12), 2752-2760. https://doi.org/10.1016/j.renene.2010.04.026.

Utomo, W. P., Wu, H., & Ng, Y. H. (2022). Quantification methodology of ammonia produced from electrocatalytic and photocatalytic nitrogen/nitrate reduction. *Energies*, *16*(1), 27. https://doi.org/10.3390/en16010027.

Verevkin, S. P., Pimerzin, A. A., Glotov, A. P., & Vutolkina, A. V. (2022). Biofuels energetics: Measurements and evaluation of calorific values of triglycerides. *Fuel*, *326*, 125101. https://doi.org/10.1016/j.fuel.2022.125101.

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., & Ruan, R. (2010). Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. *Appl Biochem Biotechnol, 162*, 1174–1186. https://doi.org/10.1007/s12010-009-8866-7.

Xue, J., Grift, T.E., Hansen, A.C. (2011). Effect of biodiesel on engine performances and emissions. *Renewable and Sustainable Energy Reviews*, *15*, 1098–1116. https://doi.org/10.1016/j.rser.2010.11.016.

You, X., Yang, L., Zhou, X., & Zhang, Y. (2022). Sustainability and carbon neutrality trends for microalgae-based wastewater treatment: A review. *Environmental Research, 209*, 112860. https://doi.org/10.1016/j.envres.2022.112860.

Zhang, K., Sun, B., She, X., Zhao, F., Cao, Y., Ren, D., & Lu, J. (2014). Lipid production and composition of fatty acids in Chlorella vulgaris cultured using different methods: photoautotrophic, heterotrophic, and pure and mixed conditions. *Annals of microbiology*, *64*(3), 1239-1246. https://doi.org/10.1007/s13213-013-0766-y.